Listing of the Claims

This listing of claims will replace all prior versions, and listings of claims in the application.

1-18. (Canceled)

19. (Currently amended) A method of producing a polynucleotide construct that encodes a fusion protein that comprises SEQ ID NO:6~~an amino acid sequence tag~~, said method comprising:

(a) obtaining a first nucleic acid molecule comprising a nucleotide sequence of interest flanked by at least a first and at least a second recombination sites that do not recombine with each other;

(b) obtaining a second nucleic acid molecule comprising: (i) at least a third and fourth recombination sites that do not recombine with each other; and (ii) ~~one or more~~ a nucleic acid sequence ~~sequences~~ which encodes SEQ ID NO:6~~an amino acid sequence tag~~; and

(c) contacting said first nucleic acid molecule with said second nucleic acid molecule under conditions favoring recombination between said first and third and between said second and fourth recombination sites, thereby producing a product polynucleotide construct; wherein said product polynucleotide construct encodes a fusion protein comprising: (i) SEQ ID NO:6~~said amino acid sequence tag~~; and (ii) the amino acid sequence encoded by said nucleotide acid sequence of interest.

20. (Original) The method of claim 19, wherein said second nucleic acid molecule further comprises a nucleic acid sequence that encodes an amino acid sequence that is capable of being cleaved by one or more proteases; and wherein said product polynucleotide construct encodes a fusion protein comprising: (i) said amino acid sequence that is capable of being cleaved by one or more proteases, flanked on one side by (ii) said amino acid sequence tag, and on the other side by (iii) the amino acid sequence encoded by said nucleotide sequence of interest.

21. (Original) The method of claim 20, wherein said amino acid sequence that is capable of being cleaved by one or more proteases is an amino acid sequence that is capable of being cleaved by enterokinase.

22-27. (Canceled)

28. (Original) The method of claim 19, wherein said second nucleic acid molecule is a vector.

29. (Original) The method of claim 19, wherein said first nucleic acid molecule is a circular nucleic acid molecule.

30. (Original) The method of claim 19, wherein said first nucleic acid molecule is a linear nucleic acid molecule.

31. (Original) The method of claim 30, wherein said first nucleic acid molecule is a PCR product.

32. (Original) The method of claim 19, further comprising inserting said product polynucleotide construct into a host cell.

33. (Original) The method of claim 20, further comprising inserting said product polynucleotide construct into a host cell.

34. (Original) The method of claim 19, wherein said second nucleic acid molecule comprises at least one additional nucleic acid sequence selected from the group consisting of a selectable marker, a cloning site, a restriction site, a promoter, an operator, an operon, a nucleotide sequence encoding a gene product which allows for negative selection, an origin of replication, a nucleotide sequence which encodes a repressor of at least one promoter, and a gene or partial gene.

35. (Currently amended) The method of claim 19, wherein said first, second, third and fourth recombination sites are selected from the group consisting of: (a) *att*B sites, (b) *att*P sites, (c) *att*L sites, (d) *att*R sites, (e) *lox* sites, (f) *psi* sites, (g) *dif* sites, (h) *cer* sites, (i)*frt* sites~~, and mutants, variants, and derivatives of the recombination sites of (a), (b), (c), (d), (e), (f), (g), (h), or (i) which retain the ability to undergo recombination~~.

36. (Original) The method of claim 19, wherein said first and said second nucleic acid molecules are combined in the presence of at least one recombination protein.

37. (Original) The method of claim 36, wherein said recombination protein is selected from the group consisting of: (a) Cre, (b) Int, (c) IHF, (d) Xis, (e) Fis, (f) Hin, (g) Gin, (h) Cin, (i) Tn3 resolvase, (j) TndX, (k) XerC, and (l) XerD.

38. (Original) The method of claim 36, wherein said recombination protein is Cre.

39-59. (Canceled)

60. (Currently amended) A method of producing a polynucleotide construct that encodes a fusion protein that comprises SEQ ID NO:6~~an amino acid sequence tag~~, said method comprising:

(a) obtaining a first nucleic acid molecule comprising a nucleotide sequence of interest;

(b) obtaining a composition comprising a second nucleic acid molecule comprising at least two topoisomerase recognition sites, at least one topoisomerase, and at least one nucleic acid sequence which encodes SEQ ID NO:6~~an amino acid sequence tag~~;

(c) mixing said first nucleic acid molecule with said composition ~~second nucleic acid molecule~~; and

(d) incubating said mixture under conditions such that said first nucleic acid molecule is inserted into said second nucleic acid molecule between said

at least two topoisomerase recognition sites, thereby producing a product polynucleotide construct; wherein said product polynucleotide construct encodes a fusion protein comprising: (i) SEQ ID NO:6~~said amino acid sequence tag~~; and (ii) the amino acid sequence encoded by said nucleotide sequence of interest.

61. (Original) The method of claim 60, wherein said second nucleic acid molecule further comprises a nucleic acid sequence that encodes an amino acid sequence that is capable of being cleaved by one or more proteases; and wherein said product polynucleotide construct encodes a fusion protein comprising: (i) said amino acid sequence that is capable of being cleaved by one or more proteases, flanked on one side by (ii) SEQ ID NO:6~~said amino acid sequence tag~~, and on the other side by (iii) the amino acid sequence encoded by said nucleotide sequence of interest.

62. (Original) The method of claim 61, wherein said amino acid sequence that is capable of being cleaved by one or more proteases is an amino acid sequence that is capable of being cleaved by enterokinase.

63-68. (Canceled)

69. (Original) The method of claim 60, wherein said second nucleic acid molecule is a vector.

70. (Original) The method of claim 60, wherein said first nucleic acid molecule is a linear nucleic acid molecule.

71. (Original) The method of claim 70, wherein said first nucleic acid molecule is a blunt-end nucleic acid molecule.

72. (Original) The method of claim 60, wherein said first nucleic acid molecule is a PCR product.

73. (Original) The method of claim 60, further comprising inserting said product polynucleotide construct into a host cell.

74. (Original) The method of claim 61, further comprising inserting said product polynucleotide construct into a host cell.

75. (Original) The method of claim 60, wherein said second nucleic acid molecule comprises at least one additional nucleic acid sequence selected from the group consisting of a selectable marker, a cloning site, a restriction site, a promoter, an operator, an operon, a nucleotide sequence encoding a gene product which allows for negative selection, an origin of replication, a nucleotide sequence which encodes a repressor of at least one promoter, and a gene or partial gene.

76. (Original) The method of claim 60, wherein said topoisomerase is a type I topoisomerase.

77. (Original) The method of claim 76, wherein said type I topoisomerase is a type IB topoisomerase.

78. (Original) The method of claim 77, wherein said type IB topoisomerase is selected from the group consisting of eukaryotic nuclear type I topoisomerase and a poxvirus topoisomerase.

79. (Original) The method of claim 78, wherein said poxvirus topoisomerase is produced by or isolated from a virus selected from the group consisting of vaccinia virus, Shope fibroma virus, ORF virus, fowlpox virus, molluscum contagiosum virus and *Amsacta moorei* entomopoxvirus.

80-103. (Canceled)

104. (Currently amended) A method of producing a polynucleotide construct that encodes a fusion protein that comprises SEQ ID NO:6~~an amino acid sequence tag~~, said method comprising:

(a) obtaining a first nucleic acid molecule comprising a nucleotide sequence of interest;

(b) obtaining a composition comprising a second nucleic acid molecule comprising (i) at least a first topoisomerase recognition site flanked by (ii) at least a first recombination site, and (iii) at least a second topoisomerase recognition site flanked by (iv) at least a second recombination site, wherein said first and second recombination sites do not recombine with each other, and (v) at least one topoisomerase;

(c) obtaining a third nucleic acid molecule comprising: (i) at least a third and fourth recombination sites that do not recombine with each other; and (ii) one or more nucleic acid sequences which encode SEQ ID NO:6~~an amino acid sequence tag~~;

(d) mixing said first nucleic acid molecule with said composition ~~second nucleic acid molecule~~;

(e) incubating said mixture under conditions such that said first nucleic acid molecule is inserted into said second nucleic acid molecule between said at least two topoisomerase recognition sites, thereby producing a first product polynucleotide construct;

(f) contacting said first product polynucleotide construct with said third nucleic acid molecule under conditions favoring recombination between said first and third and between said second and fourth recombination sites, thereby producing a second product polynucleotide construct; wherein said second product polynucleotide construct encodes a fusion protein comprising: (i) SEQ ID NO:6~~said amino acid sequence tag~~; and (ii) the amino acid sequence encoded by said nucleotide sequence of interest.

105. (Original) The method of claim 104, wherein said third nucleic acid molecule further comprises a nucleic acid sequence that encodes an amino acid sequence that is capable of being cleaved by one or more proteases; and wherein said second product polynucleotide construct encodes a fusion protein comprising: (i) said amino acid sequence that is capable of being cleaved by one or more proteases, flanked on one side by (ii) SEQ ID NO:6~~said amino acid sequence tag~~, and on the other side by (iii)the amino acid sequence encoded by said nucleotide sequence of interest.

106. (Original) The method of claim 105, wherein said amino acid sequence that is capable of being cleaved by one or more proteases is an amino acid sequence that is capable of being cleaved by enterokinase.

107-112. (Canceled)

113. (Original) The method of claim 104, wherein said second nucleic acid molecule is a vector.

114. (Original) The method of claim 104, wherein said third nucleic acid molecule is a vector.

115. (Original) The method of claim 104, wherein said first nucleic acid molecule is a linear nucleic acid molecule.

116. (Original) The method of claim 115, wherein said first nucleic acid molecule is a blunt-end nucleic acid molecule.

117. (Original) The method of claim 104, wherein said first nucleic acid molecule is a PCR product.

118. (Original) The method of claim 104, further comprising inserting said first product polynucleotide construct into a host cell.

119. (Original) The method of claim 104, further comprising inserting said second product polynucleotide construct into a host cell.

120. (Original) The method of claim 104, wherein said second and/or said third nucleic acid molecules comprises at least one additional nucleic acid sequence selected from the group consisting of a selectable marker, a cloning site, a restriction site, a promoter, an operator, an operon, a nucleotide sequence encoding a gene product which allows for negative selection, an origin of replication, a nucleotide sequence which encodes a repressor of at least one promoter, and a gene or partial gene.

121. (Currently amended) The method of claim 104, wherein said first, second, third and fourth recombination sites are selected from the group consisting of: (a) *att*B sites, (b) *att*P sites, (c) *att*L sites, (d) *att*R sites, (e) *lox* sites, (f) *psi* sites, (g) *dif* sites, (h) *cer* sites, (i) *frt* sites~~, and mutants, variants, and derivatives of the recombination sites of (a), (b), (c), (d), (e), (f), (g), (h), or (i) which retain the ability to undergo recombination~~.

122. The method of claim 104, wherein said topoisomerase is a type I topoisomerase.

123. (Original) The method of claim 122, wherein said type I topoisomerase is a type IB topoisomerase.

124. (Original) The method of claim 123, wherein said type IB topoisomerase is selected from the group consisting of eukaryotic nuclear type I topoisomerase and a poxvirus topoisomerase.

125. (Original) The method of claim 124, wherein said poxvirus topoisomerase is produced by or isolated from a virus selected from the group consisting of vaccinia virus, Shope fibroma virus, ORF virus, fowlpox virus, molluscum contagiosum virus and *Amsacta moorei* entomopoxvirus.

126. (Original) The method of claim 104, wherein said first product polynucleotide construct and said third nucleic acid molecule are combined in the presence of at least one recombination protein.

127. (Original) The method of claim 126, wherein said recombination protein is selected from the group consisting of: (a) Cre, (b) Int, (c) IHF, (d) Xis, (e) Fis, (f) Hin, (g) Gin, (h) Cin, (i) Tn3 resolvase, (j) TndX, (k) XerC, and (l) XerD.

128. (Original) The method of claim 126, wherein said recombination protein is Cre.

129-135. (Canceled)

136. (Original) A host cell comprising a polynucleotide construct that encodes a fusion protein capable of being post-translationally modified, said polynucleotide construct produced according to the method of claim 19.

137. (Original) A host cell comprising a polynucleotide construct that encodes a fusion protein capable of being post-translationally modified, said polynucleotide construct produced according to the method of claim 60.

138. (Original) A host cell comprising a polynucleotide construct that encodes a fusion protein capable of being post-translationally modified, said polynucleotide construct produced according to the method of claim 104.

139-156. (Canceled)